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# USTILOXINS, ANTIMITOTIC CYCLIC PEPTIDES FROM FALSE SMUT BALLS ON RICE PANICLES CAUSED BY *Ustilaginoidea virens*

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Ustiloxins A (**1a**), B (**1b**), C (**1c**), D (**1d**) and E (**1e**), antimitotic peptides, have been isolated from the water extract of false smut balls caused on the panicles of rice plant by a fungus *Ustilaginoidea virens*. The structure of **1b** was assigned from its spectral property and its amino acid analysis in relation to **1a** whose structure was determined previously by a conbination of X-ray crystallographic and amino acid analyses. Structures of **1c** and **1d** were elucidated by their spectroscopic data, specially based on their $^{1}H$ and $^{13}C$ NMR spectra. Bioactivities of these compounds against microtubule assembly as well as manmal, plant and fungal cells have been studied.

The false smut balls growing parasitically on panicles of rice plant (in Japanese, *Ina-kouji*) are caused by a pathogen, *Ustilaginoidea virens* Cooke, Takahashi. It has been reported that the panicles suffered from this disease has poor crops and that occasional injection of such rice plants caused poisoning to the domestic animals.

SUWA reported on the toxicity of a water extract of the smuts to rabbits[1]. YABUTA *et al.* first isolated a pigment, designated as ustilaginoidin, from the ether extract of the balls[2~4], and the structures of the compound and its homologues were determined by SHIBATA *et al.*[5,6] and KOYAMA and NATORI[7]. The compounds were, however, not the causative principles as phytotoxin or mycotoxin. We found that a water extract of the false smuts induced abnormal swelling of rice seedlings, which was initially observed as an early symptom of the "rice seedling blight", a rice plant disease caused by the pathogen *Rhizopus chinensis*[8]. The symptom was completely reproduced by rhizoxin, a phytotoxin produced by this fungus. Since rhizoxin is a potent inhibitor of microtuble assembly[9], we expected to isolate such microtubule inhibitor from the smut balls.

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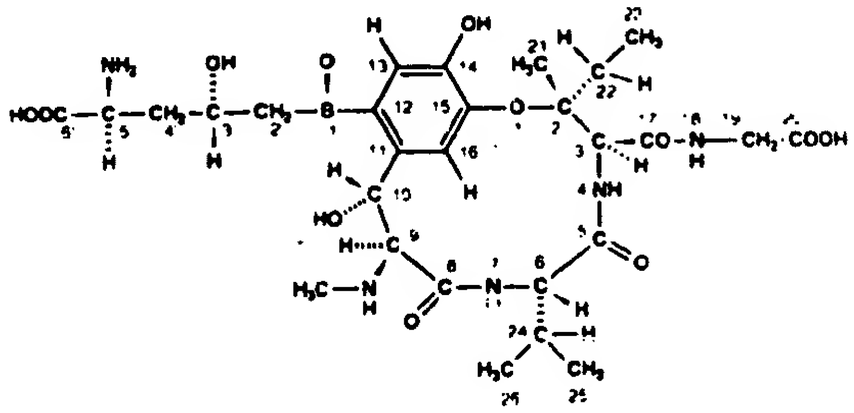
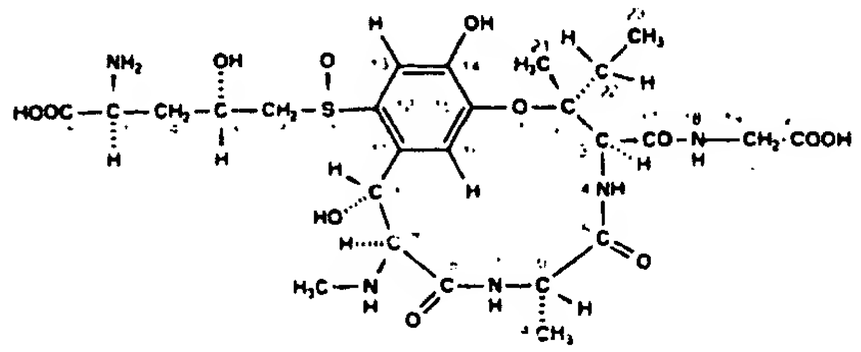
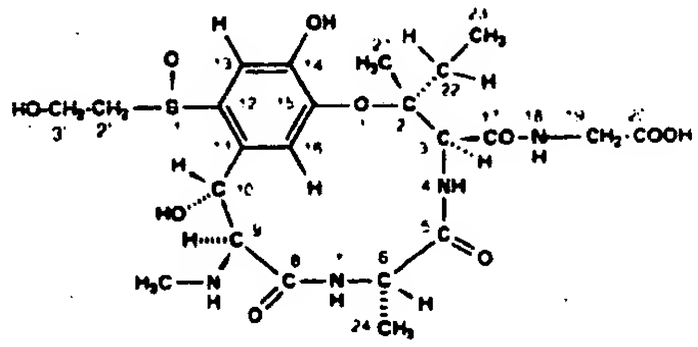
Fig. 1. Structures of ustiloxins A ~ D.



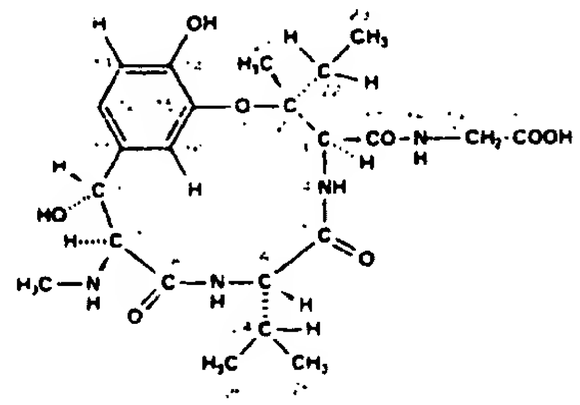
Ustiloxin A (**1a**)



Ustiloxin B (**1b**)



Ustiloxin C (**1c**)



Ustiloxin D (**1d**)

In the preceding paper[10], we reported isolation of an antimitotic cyclic peptide ustiloxin A (**1a**)† from the water extract of the false smuts. This paper deals with the isolation of additional related peptides, ustiloxins **B** (**1b**), C (**1c**), D (**1d**) (Fig. 1), and E (**1e**), and the biological activities of **1a** ~ **1e**.

## Materials and Methods

### General

UV spectra were measured on a Shimadzu apparatus, model UV-300, and the maxima were given on in nm (extinction ε). $^{1}H$ and $^{13}C$ NMR spectra were measured on a JEOL JNM GX-500 NMR spectrometer. Mass spectra were measured on a JEOL HX-110 apparatus. Thin layer chromatography were carried out on Merck Kieselgel 60F-254 plates, and HPLC was performed on a Shimadzu LC-3A apparatus.

### Amino Acid Analysis

The material was hydrolyzed by heating with 6 N HCl at 110°C for 20 hours. HCl was removed by evaporation *in vacuo*, and the resultant residue was dissolved in 0.1 N HCl and was analyzed on a Hitachi Amino Acid Analyzer, model 803.

Absolute configurations were determined by the method of NISHIMURA and KINOSHITA[11], in that each amino acid was analyzed with an ODS column after treating with *o*-phthalaldehyde in the presence of *N*-acetyl-L-cystein.

### Preparation of Microtubule Protein

Microtubule protein was prepared from porcine brains as described previously[8]. The protein concentrations were determined by the method of LOWRY *et al.*[12] using bovine serum albumin as a standard. Microtubule assembly assays were carried out in MES buffer, containing 100 mM 2-(*N*-morpholino)ethanesulfonic acid (MES), 1 mM EGTA, 0.5 mM $MgCl_2$, 1 mM 2-mercaptoethanol and 1 mM GTP (pH 6.5).

† In the preceding paper[10], the name "ustiloxin" was given for 1a, and the name of 1a is altered in this paper to ustiloxin A.

### Microtubule Assembly Assay

Microtubule assembly was monitored spectroscopically by using Shimadzu UV-300 apparatus equipped with a thermostatically regurated liquid circurator. The temperature was held at 37°C, and changes in turbidity were monitored at 400 nm. For the drug-protein study, drug dissolved in water was added to 1 ml buffer solution containing 2 mg microtubule protein.

### Rice Seedling Test

Rice seedlings were germinated in distilled water at 27°C, and were, thereafter, incubated in sample solutions for 3 days at 27°C. The shape of germs and roots of treated seedlings were compared with those grown in distilled water.

### Cytotoxicity Test

Human tumor cell line, KU-2, was kindly supplied by Dr. TAZAKI at Keio University, and the other cell lines were purchased from Immuno-Biological Laboratories, Gumma, Japanese Cancer Research Resources Cell Bank, Tokyo, and ATCC, U.S.A. The cell lines were maintained in plastic dishes (Beaton Dickson & Co., NJ) in PRMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (ICN Biomedicals Inc., CA) as the culture medium. For the cytotoxicity experiments, tumor cells (1,000 for MKN-1, MKN-7, MKN-74 and MCF-7 cells, 500 for WiDr, SW-480 and KU-2 cells, 300 for RERF-LC-MA cells, and 250 for SBC-5 cells) were seeded into wells of a 96-well culture plate containing 0.1 ml culture medium.

The plate was cultured at 37°C in humidified atmosphere of 5% $CO_2$ for 24 hours. The cells were treated with 0.1 ml of drug solutions in arrange from 0.3 nM to 10 $\mu$M and reincubated for 48 hours. After the drug exposure, the drugs were removed by washing three times with 0.2 ml of culture medium, and the cells were again incubated for 96 hours in a drug-free culture medium. Then, 50 $\mu$l of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazorium bromide: 1 mg/ml) was added, and incubation was continued for further 4 hours. Finally, culture medium was discarded and 150 $\mu$l DMSO was added. After vigorous shaking of the plate for 5 minutes, optical density of each culture was measured at 540 nm.

Four wells were used for each drug concentration. In the control culture, tumor cells grew exponentially during the incubation period. Ustiloxins were dissolved in a small amount of DMSO and diluted with the culture medium. The $IC_{50}$ values were determined by plotting the logarithumus of the drug concentrations *versus* growth rates of treated cells (percentage of control).

## Results

### Isolation and Purification

The isolation procedure of ustiloxins A ~ E (**1a** ~ **1e**) is illustrated in Fig. 2. The water extract of the smut balls was resolved by ODS column into two parts; fractions eluted with water in which most of the extract was eluted, and the fractions eluted with 20% aqueous methanol containing ustiloxins. The latter portion was separated by succsessive chromatographies as shown in Fig. 2, by monitoring the activity to cause abnormal swelling of rice seedling roots (see the part of biological activity) for **1a** and **1b**, and by detection on TLC for **1c** ~ **1e**. The final purification of **1a** was performed by recrystallization from aqueous methanol[10] and **1b** ~ **1e** were purified by HPLC using an ODS column.

### Properties

Compounds **1b** ~ **1e** were obtained as colorless powders and were, except **1d**, Ninhydrin positive on TLC. They, as well as **1a**, are all easily soluble in water, and are hardly soluble in organic solvents. The physico-chemical properties of these compounds are shown in Table 1. The $^1$H NMR data of **1b** ~ **1e** are shown in Table 2, comparing with the data of ustiloxin A (**1a**). The signal assignments were made based on chemical shifts, $^1$H-$^1$H COSY experiment. The $^{13}$C NMR data of compounds **1b** ~ **1d** are shown in

Fig. 2. A diagram of isolation procedure.

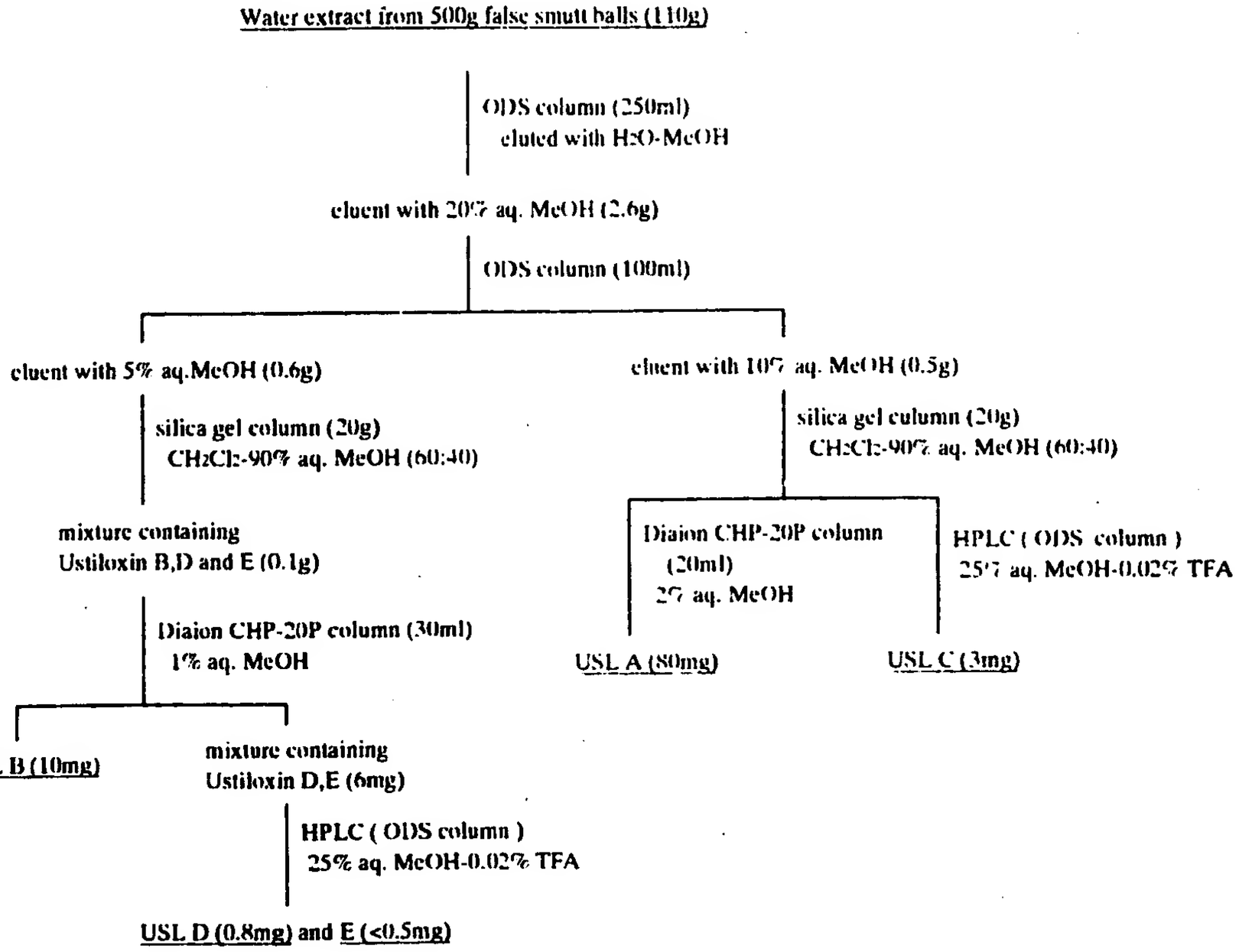



Table 1. Physico-chemical properties of ustiloxins.

| | Ustiloxin A | Ustiloxin B | Ustiloxin C |
|---|---|---|---|
| Molecular formula | $C_{28}H_{43}N_5O_{12}S$ | $C_{26}H_{39}N_5O_{12}S$ | $C_{23}H_{34}N_4O_{10}S$ |
| Appearance | Colorless needle | Colorless powder | Colorless powder |
| UV $\lambda_{max}^{H_2O}$ nm ($\varepsilon$) | 207 (15,000), 253 (7,300), 290 (3,800) | 213 (13,900), 252 (5,000), 290 (2,500) | 216 (12,400), 253 (5,000), 290 (2,900) |
| FAB-MS ($m/z$) (M+H) | 674 | 646 | 559 |
| HRFAB-MS ($m/z$) (M+H) | | | |
| Found: | 674.2730 | 646.2390 | 559.2085 |
| Calcd: | 674.2718 | 646.2394 | 559.2073 |
| $[\alpha]_D$ | +14.5° ($c$ 0.55, $H_2O$) | +14.1° ($c$ 0.50, $H_2O$) | — |

| | Ustiloxin D | Ustiloxin E |
|---|---|---|
| Molecular formula | $C_{23}H_{34}N_4O_8$ | $C_{28}H_{41}N_5O_{11}S$ |
| Appearance | Colorless powder | Colorless powder |
| UV $\lambda_{max}^{H_2O}$ nm ($\varepsilon$) | 208 (10,100), 227 (8,700), 287 (2,800) | 213 (13,900), 252 (5,000), 290 (4,900) |
| FAB-MS ($m/z$) (M+H) | 495 | 656 |
| HRFAB-MS ($m/z$) (M+H) | | |
| Found: | 495.2401 | 656.2622 |
| Calcd: | 495.2455 | 656.2601 |
| $[\alpha]_D$ | — | — |

Table 3 in which the signal assignments were obtained from the comparison of the chemical shifts values with those for **1a**, $^1H$-$^{13}C$ COSY and heteronuclear multiple-bond $^1H$-$^{13}C$ correlation spectroscopy (HMBC).

Table 2. $^1$H NMR data of ustiloxin A, B, C, D and E in $D_2O$.

| Proton | Chemical shift ($\delta$), multiplicity and coupling constant (in Hz) | | | | |
|---|---|---|---|---|---|
| | Ustiloxin A | Ustiloxin B | Ustiloxin C | Ustiloxin D[a] | Ustiloxin E[a] |
| 3-H | 4.83 (1H, s) | 4.56 (1H, s) | 4.66 (1H, s) | 4.78 (1H, s) | 4.63 (1H, s) |
| 6-H | 4.13 (1H, d, $J_{6,24}=10.0$) | 4.37 (1H, q, $J_{6,24}=7.0$) | 4.40 (1H, q, $J_{6,24}=7.0$) | 4.00 (1H, d, $J_{6,24}=9.5$) | 4.14 (1H, d, $J_{6,24}=7.0$) |
| 9-H | 4.28 (1H, d, $J_{9,10}=10.0$) | 4.00 (1H, d, $J_{9,10}=10.0$) | 4.17 (1H, d, $J_{9,10}=10.0$) | 3.84 (1H, d, $J_{9,10}=9.6$) | 3.86 (1H, s) |
| 9-$NCH_3$ | 2.77 (3H, s) | 2.60 (3H, s) | 2.70 (3H, s) | 2.68 (3H, s) | 2.61 (3H, s) |
| 10-H | 4.96 (1H, d, $J_{10,9}=10.0$) | 4.80 (1H, d, $J_{10,9}=10.0$) | 4.92 (1H, d, $J_{10,9}=10.0$) | 4.71 (1H, d, $J_{10,9}=9.6$) | |
| 12-H | | | | 7.13 (1H, dd, $J_{12,13}=8.3$, $J_{12,16}=2.0$) | |
| 13-H | 7.61 (1H, s) | 7.47 (1H, s) | 7.52 (1H, s) | 7.01 (1H, d, $J_{13,12}=8.3$) | 7.74 (1H, s) |
| 16-H | 7.11 (1H, s) | 7.28 (1H, s) | 7.38 (1H, s) | 6.96 (1H, d, $J_{16,12}=2.0$) | 7.49 (1H, s) |
| 19-H | 3.79 (2H, s) | 3.70 (1H, d, $J_{19,19}=17.0$), 3.76 (1H, d, $J_{19,19}=17.0$) | 3.95 (1H, d, $J_{19,19}=17.0$), 4.03 (1H, d, $J_{19,19}=17.0$) | 3.93 (2H, s), | 3.80 (1H, d, $J_{19,19}=16.0$), 3.85 (1H, d, $J_{19,19}=16.0$) |
| 21-H | 1.77 (3H, s) | 1.68 (3H, s) | 1.69 (3H, s) | 1.63 (3H, s) | 1.31 (3H, s) |
| 22-H | 1.73 (1H, dq, $J_{22,22}=14.2$, $J_{22,23}=7.2$), 2.24 (1H, dq, $J_{22,22}=14.2$, $J_{22,23}=7.2$) | 1.63 (1H, dq, $J_{22,22}=14.0$, $J_{22,23}=7.2$), 2.05 (1H, dq, $J_{22,22}=14.0$, $J_{22,23}=7.2$) | 1.66 (1H, dq, $J_{22,22}=14.0$, $J_{22,23}=7.5$), 2.07 (1H, dq, $J_{22,22}=14.0$, $J_{22,23}=7.5$) | 1.63 (1H, dq, $J_{22,22}=14.8$, $J_{22,23}=7.4$), 2.06 (1H, dq, $J_{22,22}=14.8$, $J_{22,23}=7.4$) | 1.72 (1H. dq, $J_{22,22}=14.8$, $J_{22,23}=7.5$), 1.83 (1H, dq, $J_{22,22}=14.8$, $J_{22,23}=7.5$) |
| 23-H | 1.09 (3H, dd, $J_{23,22}=7.2$, 7.2) | 0.92 (3H, dd, $J_{23,22}=7.2$, 7.2) | 0.92 (3H, dd, $J_{23,22}=7.5$, 7.5) | 1.02 (3H, dd, $J_{22,23}=7.4$, 7.4) | 0.75 (3H, dd, $J_{23,22}=7.5$, 7.5) |
| 24-H | 1.92 (1H, m, $J_{24,6}=10.0$, $J_{24,25}=7.0$, $J_{24,26}=7.0$) | 1.14 (3H, d, $J_{24,6}=7.0$) | 1.15 (3H, d, $J_{24,6}=7.0$) | 1.78 (1H, m, $J_{24,6}=9.5$, $J_{24,25}=6.7$, $J_{24,26}=6.7$) | 1.98 (1H, m, $J_{24,6}=7.0$, $J_{24,25}=7.0$) |
| 25-H | 0.80 (3H, d, $J_{25,24}=7.0$) | | | 0.69 (3H, d, $J_{25,24}=6.7$) | 0.80 (3H, d, $J_{25,24}=7.0$) |
| 26-H | 0.88 (3H, d, $J_{26,24}=7.0$) | | | 0.77 (3H, d, $J_{26,24}=6.7$) | |
| 2'-H | 3.04 (1H, dd, $J_{2',2'}=13.6$, $J_{2',3'}=3.0$), 3.33 (1H, dd, $J_{2',2'}=13.6$, $J_{2',3'}=10.0$) | 2.96 (1H, dd, $J_{2',2'}=13.4$, $J_{2',3'}=2.8$), 3.30 (1H, dd, $J_{2',2'}=13.4$, $J_{2',3'}=10.0$) | 2.94 (1H, ddd, $J_{2',2'}=14.0$, $J_{2',3'}=4.0$, 4.0), 3.37 (1H, ddd, $J_{2',2'}=14.0$, $J_{2',3'}=5.5$, 9.5) | | 2.72 (1H, dd, $J_{2',2'}=13.8$, $J_{2',3'}=2.0$), 3.16 (1H, dd, $J_{2',2'}=13.8$, $J_{2',3'}=9.4$) |
| 3'-H | 4.39 (1H, m, $J_{3',2'}=3.0$, 10.0, $J_{3',4'}=3.0$, 10.0) | 4.29 (1H, m, $J_{3',2'}=2.8$, 10.0, $J_{3',4'}=3.0$, 10.0) | 3.97 (1H, ddd, $J_{3',3'}=12.5$, $J_{3',2'}=4.0$, 5.5), 4.01 (1H, ddd, $J_{3',3'}=12.5$, $J_{3',2'}=4.0$, 9.5) | | 4.25 (1H, m) |
| 4'-H | 2.12 (1H, ddd, $J_{4',3'}=3.0$, $J_{4',4'}=15.0$, $J_{4',5'}=8.0$), 2.22 (1H, ddd, $J_{4',3'}=10.0$, $J_{4',4'}=15.0$, $J_{4',5'}=4.0$) | 2.01 (1H, ddd, $J_{4',3'}=3.0$, $J_{4',4'}=15.0$, $J_{4',5'}=8.0$), 2.12 (1H, ddd, $J_{4',3'}=10.0$, $J_{4',4'}=15.0$, $J_{4',5'}=4.0$) | | | 1.98 (1H, m), 2.04 (1H, m) |
| 5'-H | 4.01 (1H, dd, $J_{5',4'}=4.0$, 8.0) | 3.90 (1H, dd, $J_{5',4'}=4.0$, 8.0) | | | 3.86 (1H, m), 9.60 (1H, s) |

[a] The data are for a TFA (trifluoroacetic acid) salt.

Table 3. $^{13}C$ NMR data of ustiloxin A, B, C, and D.

| Carbon | Chemical shift ($\delta$) | | | | Carbon | Chemical shift ($\delta$) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ustiloxin A | Ustiloxin B | Ustiloxin C | Ustiloxin D[a] | | Ustiloxin A | Ustiloxin B | Ustiloxin C | Ustiloxin D[a] |
| C-2 | 87.2 | 84.5 | 85.7 | 85.6 | C-17 | 170.3 | 170.6 | 169.6 | 163.6 |
| C-3 | 59.6 | 60.3 | 58.3 | 59.1 | C-19 | 43.8 | 44.2 | 40.2 | 41.5 |
| C-5 | 171.0 | 172.7 | 170.9 | 170.0 | C-20 | 176.3 | 176.9 | 171.9 | 178.0 |
| C-6 | 60.1 | 50.0 | 48.4 | 59.9 | C-21 | 21.1 | 22.2 | 20.8 | 21.1 |
| C-8 | 166.4 | 169.4 | 164.9 | 163.3 | C-22 | 32.1 | 31.7 | 29.8 | 32.2 |
| C-9 | 66.7 | 68.2 | 65.0 | 68.7 | C-23 | 7.8 | 8.4 | 6.7 | 7.8 |
| 9-$NCH_3$ | 32.1 | 33.0 | 30.6 | 32.4 | C-24 | 28.7 | 15.7 | 14.2 | 28.9 |
| C-10 | 73.9 | 74.7 | 72.1 | 72.8 | C-25 | 17.9 | | | 17.8 |
| C-11 | 128.0 | 129.0 | 127.3 | 127.8 | C-26 | 18.3 | | | 18.2 |
| C-12 | 136.4 | 137.1 | 136.9 | 118.8 | C-2′ | 64.8 | 65.1 | 59.2 | |
| C-13 | 114.4 | 114.4 | 112.7 | 107.6 | C-3′ | 63.8 | 64.2 | 54.4 | |
| C-14 | 152.2 | 153.2 | 151.7 | 153.4 | C-4′ | 36.7 | 37.1 | | |
| C-15 | 146.0 | 146.3 | 144.3 | 142.4 | C-5′ | 52.7 | 53.2 | | |
| C-16 | 124.2 | 124.6 | 123.3 | 123.9 | C-6′ | 174.4 | 174.8 | | |

[a] The data are for a TFA (trifluoroacetic acid) salt.

## Structures

The structures of ustiloxins **B** (**1b**), C (**1c**) and D (**1d**) were determined spectroscopically by comparison with the data of ustiloxin A (**1a**) whose structure was established by a X-ray crystallographic and an amino acid analysis[10].

Ustiloxin B gave a HRFAB-MS spectrum agreeing with the formula $C_{26}H_{39}N_5O_{12}S$ which is a $C_2H_4$ unit less than that of **1a**. The $^1H$ and $^{13}C$ NMR spectra of **1b** lacked the signals assigned for the two methyls of the valine residue (positions 25 and 26) in **1a**, and instead exhibited signals assigned for a methyl group at position 24. These facts suggested that a valine unit present in **1a** is replaced by an alanine in **1b**. An acid hydrolysis of **1b** indeed gave a L-alanine and a glycine as the detectable amino acids. The substitution pattern on the phenyl ring was shown to be the same in both compounds by their $^1H$ NMR spectra.

Ustiloxin C gave a HRFAB-MS spectrum agreeing with the formula $C_{23}H_{34}N_4O_{10}S$ which is $C_3H_5NO_2$ less than that of **1b**. The $^1H$ and $^{13}C$ NMR spectra of **1c**, as in the case of **1b**, exhibited a methyl signal assigned for position 24, indicating that the partial structure of **1c** is also composed of an alanine moiety. Remarkable difference between the NMR spectra of **1b** and **1c** was that the spectra of **1c** lacked the signals due to the $C(4')H_2{\sim}C(5')H(NH_2){\sim}C(6')OOH$ moiety present in **1b** (see Tables 2 and 3). The presence of the $C(2')H_2{\sim}C(3')H_2{\sim}OH$ moiety in **1c** was verified by $^1H$-$^1H$ and $^1H$-$^{13}C$ COSY, and $^1H$-$^{13}C$ HMBC between 2′-$H_2$ and C-12. The substitution pattern on the phenyl ring was determined by $^1H$ NMR spectrum by comparison with those of **1a** and **1b**.

Ustiloxin D (**1d**), the simplest homologue of ustiloxins, gave a HRFAB-MS spectrum agreeing with the formula $C_{23}H_{34}N_4O_8$. Its NMR spectra indicated that it is a tetrapeptide consist of a 3-(3,4-oxyphenyl)-3-hydroxy-*N*-methyl-alanine, a valine, a 3-oxyisoleucine and a glycine moieties which are common with the structure of **1a**, but it carry no substituent at C-12 present in **1a**. The substitution pattern on the phenyl ring was established by $^1H$-$^1H$ COSY experiment. Compound **1d** should supposedly be the biosynthetic precursor of **1a**.

Though the structure of ustiloxin E has not been determined because of the shortage of the material, its spectral data suggested that it has a structure slightly modified from that of **1a**, and that it could be a metabolite of **1a**.

## Biological Activities

Ustiloxin A (**1a**) and B (**1b**) induced such abnormal swelling of rice seedling roots as shown in

Fig. 3. Effect of ustiloxin A on the rice seedling root.

Drug concentration: a; none (control), b; 100 μg/ml, c; 10 μg/ml, d; 1 μg/ml.

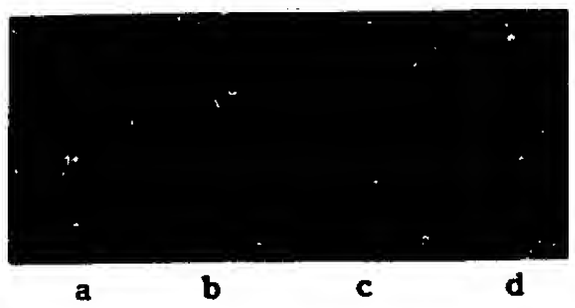
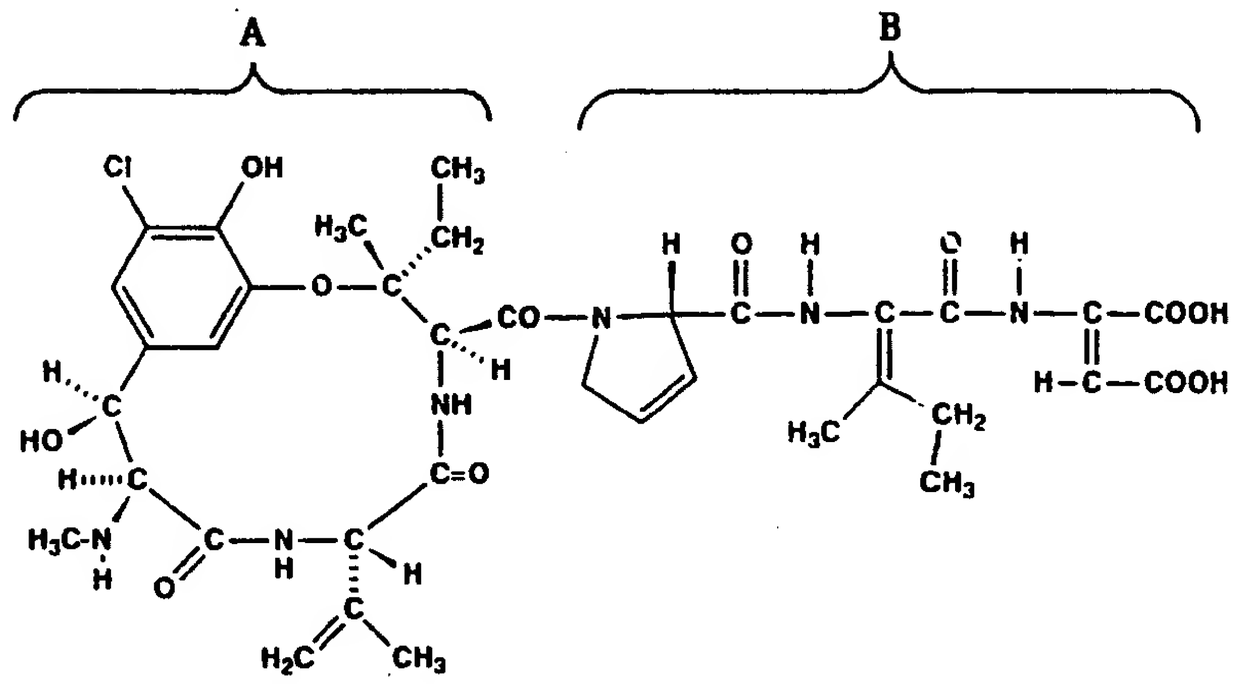


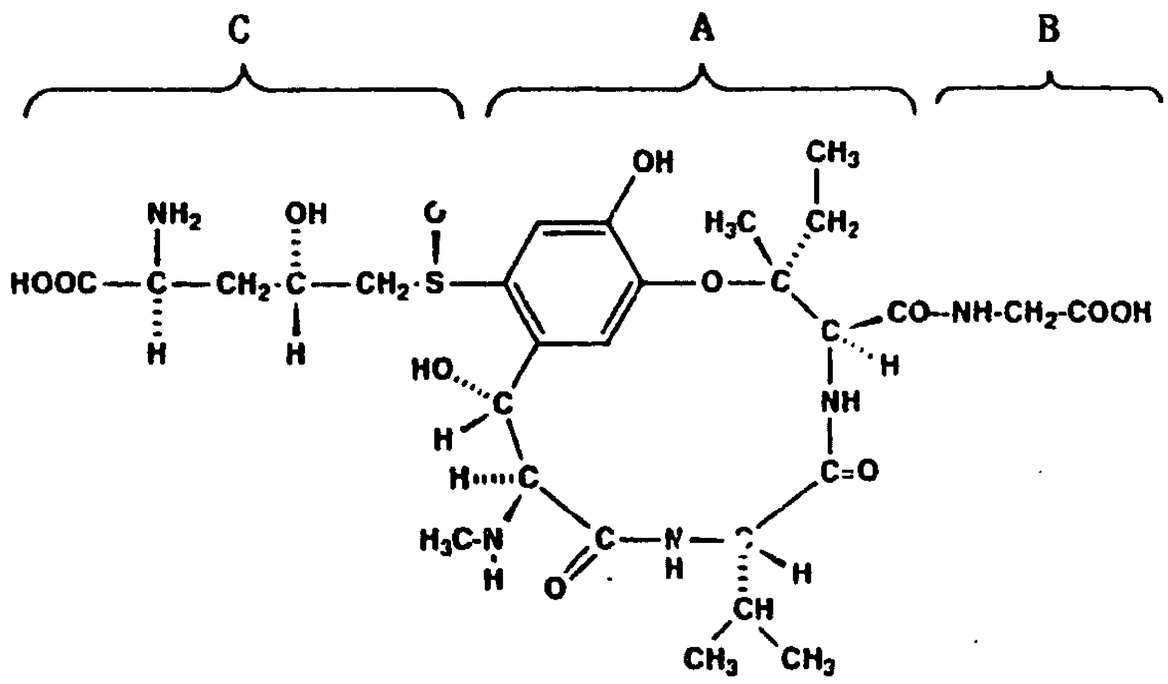

Table 4. Cytotoxity of ustiloxins in human tumor cell lines.

| Cell | Origin | $IC_{50}$ (μM) | |
|---|---|---|---|
| | | Ustiloxin A | Ustiloxin B |
| MKN-1 | Stomach | 3.22 | 4.42 |
| MKN-7 | Stomach | 4.21 | 7.81 |
| MKN-74 | Stomach | 5.19 | 17.65 |
| RERF-LC-MA | Lung | 5.60 | 13.64 |
| SBC-5 | Lung | --- | 0.39 |
| MCF-7 | Breast | 0.86 | 2.14 |
| WiDr | Colon | 4.68 | 8.99 |
| SW-480 | Colon | 6.08 | 3.95 |
| KU-2 | Kidney | 4.43 | 3.95 |

Fig. 4. A display of the partial structures of phomopsin A and ustiloxin A.




Phomopsin A




Ustiloxin A

Fig. 3. This would reflect the mode of action of these compounds that interfere with the formation of cell skeleton by inhibiting microtubule assembly, as previously demonstrated with rhizoxin[8,9]. Such effect has not been tested for **1c**~**1e** because of their limited sample scales.

The inhibitory effects of ustiloxins A~D (**1a**~**1d**) on the polymerization of microtubules proteins were tested. Ustiloxins exhibited potent activities, and their $IC_{50}$ values were determined to be 0.7, 2.8, 4.4, and 6.6 $\mu$M for **1a**~**1d**, respectively. Ustiloxin E (**1e**) did not show significant effect on microtubule assembly. The details of the interaction between ustiloxins and tubulin will be reported elsewhere.

Ustiloxins A and B inhibited the mitosis of a variety of human tumor cell lines as shown in Table 4. In contrast, they showed no growth inhibitory effect on both bacteria and fungi (data not shown) similar to the other two antimitotic peptides, phomopsin A[13,14] and dolastatin 10[15].

Toxicity of ustiloxins against animals was tested with mice. Single injection of a crude mixture of ustiloxins and of ustiloxin A (**1a**) caused acute necrosis of isolated hepatocites and renal tubular cells followed by mitotic arrest and abnormal mitosis resembling that caused by colchicine. Serial injections caused relatively mild but definite liver and kidney leisons. These leisons were the same as those observed in "lupinosis" caused by phomopsin A. The detailed results are reported elsewhere[16].

## Discussion

Ustiloxins are unique tetrapeptides containing a 13-membered ring, including an ether linkage. Their structures are closely related to that of phomopsin A, a mycotoxic hexapeptide to cause lupinosis[13].

Biological property of ustiloxins also resemble to that of phomopsin A, such that both cause similar micotoxicosis and strongly inhibit polymerization of brain tubulin, but show no activity to fungi. That is, they are biologically acting in a very similar fashion. On the other hand, as is seen in Fig. 4, a common structural element present in both ustiloxin A and phomopsin A lie in the partial structure A, except the absolute configurations at C-10, and their structures differ largely in the parts B and C

These facts led us to assume that the part A should be the fundamental structural element for their interaction with tubulin. Newly found ustiloxin D (**1d**), indeed, showed significant anti-tubulin activity. These findings prompt us to study their essential structural factor(s) to interact with tubulin, which can lead to a variety of derivatives for biochemical and biomedical purposes.

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